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AGAINST Q-FEVER. REPORT 2. SKIN AND ORAL ROUTES OF IMMUNIZATION

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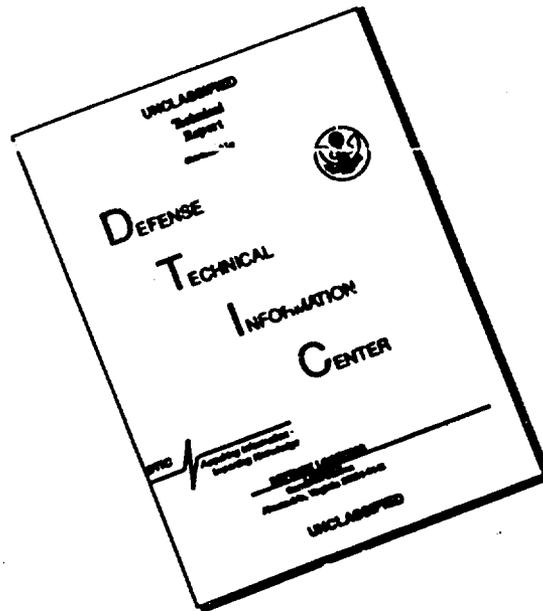
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**EXPERIENCE ON MASS IMMUNIZATION OF HUMAN BEINGS WITH THE M-44 LIVE VACCINE  
AGAINST Q-FEVER. REPORT 2. SKIN AND ORAL ROUTES OF IMMUNIZATION**

[Following is the translation by V. A. Genig, N.F. Gamaleya  
Institute of Epidemiology and Microbiology, AMN USSR, Moscow,  
published in the Russian-language periodical Voprosy Virusologii  
(Problems of Virology) No 6, 1965, pages 703--707. It was sub-  
mitted on 1 Jul 1964. Translation performed by Sp/7 Charles T.  
Ostertag, Jr.]

The results of experimental investigations on guinea pigs showed that the creation of an expressed immunity is possible by way of various methods of immunization with the live M-44 vaccine, including cutaneous application and administration of the vaccine through the mouth. Here it was established that the minimum immunizing doses of vaccine for the cutaneous and oral methods of administration are 100--1000 times greater than for subcutaneous vaccination, which is one of the most effective methods of immunizing laboratory animals. The results of the tests also showed that during the cutaneous method of vaccination the most expressed immunological effect was observed with the application of concentrated vaccines, containing no less than  $10^8$ -- $10^9$  MIDE, and when the skin had a large number of scarifications, and during the oral method -- when a guarantee was made for the maximum possible absorption of the rickettsiae through the mucous membrane of the oral cavity.

After the cutaneous and oral administration of vaccine containing  $10^8$ -- $10^9$  MIDE it was characteristic for guinea pigs that a brief rickettsemia develops (1--3 days) in the 1st week after immunization. The rickettsias take root in the regional lymph nodes and the spleen for 20 days, there is an absence of a temperature reaction in the majority of animals and the appearance of a weakly expressed local reaction following cutaneous application in the form of a light swelling and reddening along the scarifications.

Complement fixing antibodies appeared in the sera of the guinea pigs in 10--14 days and reached the highest level after 25--30 days following immunization (1:40--1:160). Then the antibody titer gradually lowered and after 6 months antibodies were detected in low titers (1:5--1:10) in individual animals.

A protective effect developed in the animals already in 5--7 days after a single inoculation, but an immunity of a high intensity (to 100 000 infecting doses of a virulent culture) was detected after 1--2 months and an expressed immunity to 1000--10 000 infecting doses of a virulent culture of Rickettsia burneti was preserved for the next 8--10 months (period of observation).

In accordance with the experimental data obtained in the tests on animals the first cutaneous and oral inoculations of human beings were

carried out with the application of inoculation doses which were 100--1000 times greater than the effective subcutaneous inoculation dose and corresponded to  $10^7$ -- $10^8$  and  $10^8$ -- $10^9$  MIDE.

#### Materials and Methods

The live vaccine for cutaneous and oral application was a lyophile dried 50% suspension of infected vitelline sacks containing a large quantity of rickettsia of the M-44 vaccine strain. The suspension from the infected vitelline sacks was prepared on skimmed sterile milk.

The infection titer of the vaccine on chick embryos corresponded to  $10^{-9}$ -- $10^{-10}$ . The vaccine was bacteriologically sterile and harmless.

For the cutaneous application the dry vaccine was diluted with physiological solution and one drop was applied to each of two sectors of the shoulder which had been preliminarily treated with alcohol or ether. Using a vaccination quill we made 3 cross shaped incisions up to 1 cm in length through each drop of vaccine. These were at a distance of 0.3--0.4 cm from each other. The incisions were made at a depth at which the blood came out in fine drops. The vaccine was rubbed into the incisions and allowed to dry completely.

The first group of persons (473 men) were inoculated with a vaccine diluted 1:10 (in respect to the initial weight of the vitelline sack), and the second group (291 men) -- with a vaccine in a dilution of 1:2, which fixed on the scarified skin better and dried faster. One inoculating dose of cutaneous vaccine contained no less than  $10^7$ -- $10^8$  MIDE.

For oral application the dry vaccine was preliminarily diluted in 2.5 ml of milk, which corresponded to a vaccine dilution of 1:10. A dose of 1 ml of vaccine was administered orally on a lump of sugar or in 20 ml of milk, and it was compulsory to rinse the mouth with the vaccine in order to ensure the maximum absorption of the vaccine through the mucous membrane of the oral cavity. For oral administration one inoculation dose of the vaccine contained  $10^8$ -- $10^9$  MIDE.

Cutaneous inoculations were performed on 764 men, oral on 65. Prior to inoculation the number of serum positive persons in the various groups fluctuated from 4 to 29%. The inoculations were carried out in regions which were unsafe for Q-fever on workers from tanneries, students from factory-workshop schools at meat-combines, university students who were getting industrial experience at meat-combines and workers from rickettsiosis laboratories. The ages of the inoculated persons fluctuated from 14 to 55 years. Observations were carried out for 10 days from the time of vaccination. This was done by taking into consideration objective and subjective data and then by questioning after 1--3 months after the inoculation. All told under observation were 405 men who had been inoculated subcutaneously and 56 who had received the vaccine orally.

The immunological effectiveness of the vaccine was evaluated based on the presence and level of specific antibodies. These were determined in the complement fixation reaction which was set up by the generally accepted method in the cold.

The serological investigations were carried out after 21 days and 1--8 months after the inoculation. All told 546 sera were investigated.

#### Results

During the cutaneous application of the vaccine both in the persons who had received the vaccine in a dilution of 1:10 and in those immunized with the vaccine in a dilution of 1:2 no general inoculation reactions with an increase of temperature were observed. Five of the 405 investigated complained on the 2nd, 3rd and 5th day of a 1-day feeling of poor health or headache. These reactions passed rapidly and were not accompanied by an inability to work.

By the third day after the vaccination a fine nodular swelling and reddening appeared along the scarifications at the site of administration of the vaccine. These were more expressed on the 4--5th day. After 2 days the cutaneous reactions faded away rapidly, leaving only traces of desquamation. No enlargement of the regional lymph nodes was noted in the inoculated persons.

With the administration of vaccine in a dilution of 1:10 a local reaction was observed in 48.4--51% of those inoculated. Nodular swelling and reddening often appeared only along individual incisions. In the inoculated persons of the second group, who received the vaccine in a dilution of 1:2, the local reaction appeared in the same period as the reaction was observed during immunization of vaccine in a dilution of 1:10, however, the local reactions were observed in 80% of the inoculated persons in the second group and in a number of cases the local reactions were more expressed.

In the persons who reacted positively in the serum immunological reaction prior to inoculation the local reaction was manifested in the absolute majority after administration of vaccine in a dilution of 1:2, and was the same in positively and negatively reacting persons to the administration of vaccine in a dilution of 1:10.

In persons with serum positive reactions we did not observe expressed allergic reactions, including the subjective symptoms of an inoculation reaction.

As a result of serological investigations, conducted after 21 days and 1--3 months after the inoculation, a clear correlation was established between the local inoculation reaction developing and the ensuing production of specific antibodies.

Thus, with the application of vaccine in a dilution of 1:10 a local reaction was manifested in 48--51% of those inoculated, and serum positive reactions after 1--3 months following inoculation were noted in 30% of

the cases. During the immunization with vaccine in a dilution of 1:2 the local reactions were recorded in 80% of those inoculated, and correspondingly the number of serum positive reactions increased up to 85% (Table 1).

In the inoculated group in which the local reaction was clearly manifested 87% of positive serological reactions was recorded with an average antibody titer of 1:46. In the absence of a local reaction specific antibodies were detected in 79% of those inoculated with an average titer of 1:30.

The results of the serological investigations showed that the vaccine in a dilution of 1:2 guaranteed a more expressed immunological effect than the vaccine in a dilution of 1:10.

After a single cutaneous administration of the vaccine in a dilution of 1:2, specific antibodies were detected in the majority of inoculated persons after 21 days -- 1 month following inoculation (80--91%). However, the titers of antibodies in this period of investigation were low and fluctuated within 1:5 -- 1:20. Higher antibody titers were reached after 2--3 months and fluctuated within 1:5 -- 1:640. After 2 months following inoculation the number of complement fixation reactions with an average titer of antibodies of 1:52 was recorded in 85% of those inoculated, and after 3 months with an average titer of 1:72- in 76.1%. After 8 months antibodies were preserved in 53% of those inoculated with an average titer of 1:20 (Table 2).

In persons with a serum positive reaction prior to inoculation the serum immunological shift was manifested in different ways and apparently depended of the level of intensity of immunity in the inoculated persons prior to vaccination.

Thus, with low serological indices prior to vaccination (1:5--1:10) we noted a lowering of the titer of antibodies to negative results, an absence of a serum immunological shift or an increase of titers by 2--3 times. With average and high serological indices (1:20--1:80 and higher) we noted an insignificant serum immunological shift or it was absent.

In a comparison of the serological indices in persons inoculated by the cutaneous and subcutaneous methods it turned out that after cutaneous immunization with vaccine in a dilution of 1:2 the antibody titers in the inoculated persons were somewhat more expressed than after subcutaneous administration of optimum doses of vaccine, and were preserved more persistently at a high level for a period of 3 months from the time of vaccination.

In this manner, during cutaneous application the live M-44 vaccine in large doses, containing on an order of  $5 \times 10^7$ -- $10^8$  MIDE, turned out to be mildly reactogenic and at the same time guaranteed a well expressed immunological effect. In persons with serum positive reactions prior to inoculation expressed allergic reactions were not observed after vaccination with the live vaccine. This testifies to the feasibility of carrying out mass

inoculations with live Q-fever vaccine without a preliminary selection based on the serum immunological reaction.

After a single administration of live vaccine through the mouth, no expressed local and general reactions (pain in the throat, dyspepsia phenomena) were observed in the inoculated persons.

As the serological investigations showed, the development of post-vaccinal immunity against Q-fever following the oral administration of M-44 vaccine was also accompanied by the formation of complement fixing antibodies, which were detected for 2--3 months (period of observation) in 80.3% of those inoculated (see Table 2). Based on expressiveness and level the antibody titers after oral immunization were somewhat lower than following cutaneous vaccination with an optimum inoculation dose, and approximately the same with the serological indices obtained during subcutaneous immunization.

Thus, the results of the first observations of oral immunization of human beings showed the principle possibility of vaccination with the live M-44 vaccine through the mouth. It is necessary to have a further study of the expressiveness and duration of postvaccinal immunity after oral vaccination during remote periods, and also to develop methods for preparing special tablets, which by oral application would guarantee the reproduction of an immunity against Q-fever.

#### Conclusions

1. With cutaneous application the live M-44 Q-fever vaccine turned out to be mildly reactogenic. The symptoms of a general reaction were weakly expressed and observed rarely (1.3%).

2. Based on immunological effectiveness the cutaneous method of vaccination with the live M-44 vaccine in the corresponding vaccine dose is not inferior to the subcutaneous method. Along with this it is a simpler method of immunization and this makes it possible to recommend the cutaneous live Q-fever vaccine for practical utilization in the mass inoculation of threatened contingents.

3. Carrying out the mass immunization of human beings with the live Q-fever vaccine is possible without the preliminary selection based on serum immunological reactions.

4. The results of the tests carried out on the harmlessness and immunological effectiveness of the live vaccine when it is administered by the oral route testify to the favorable outlook for putting into practice the oral application of the live Q-fever vaccine.

Table 1

Reactogenic properties of the live M-44 vaccine during cutaneous application of the vaccine

Dose of vaccine (dilution)	CFR prior to inoculation	Number inoculated	Number of General reactions Poor health headache	Number of local reactions		CFR after 1--2 months after inoculation	
				abs.	%	Number investigated	Number of pos. sera
1:10	Negative	219	1	106	48.4	84	25(29.8%)
	Positive	35	1	18	51	11	5
1:2	Negative	133	2	107	80	134	114(85%)
	Positive	18	1	16		14	12

Table 2

Immunogenic properties of the live M-44 vaccine during cutaneous and oral application

Method of administration and dose of vaccine	Period of investigation (in months)	Number of sera investigated	Number of positive sera		Average antibody titer	Titer in the CFR	
			abs.	%		1:5--1:20	1:40--1:640
Cutaneous 1:10	1	20	6	30	1:30		
	2--3	84	25	29.8	1:10	96.0	4.0
	6--8	96	32	33.7	1:10	96.0	4.0
1:2	up to 1	15	12		1:14	83.3	16.7
	1--1.5	69	55	80	1:30	67.2	32.8
	2	71	61	85.9	1:52	44.2	55.8
	3 8	42 84	32 45	76.1 53.5	1:72 1:20	47.0 86.6	53.0 13.4
Oral 1:10	2--3	56	45	80.3	1:30	63.6	39.4